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Ginsenoside Content of North American Ginseng (*Panax quinquefolius* L. Araliaceae) in Relation to Plant Development and Growing Locations

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Abstract : North American ginseng (*Panax quinquefolius* L.) was analysed for total ginsenosides and ten major ginsenosides (R_0, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, pseudoginsenoside F_{11}, and gypenoside XVII), and variations in ginsenoside content with age of plant (over a four-year-period) and geographic location (Ontario versus British Columbia) were investigated. In the roots the total ginsenoside content increased with age up to 58-100 mg · g^{-1} dry weights in the fourth year, but in leaves it remained constant over time. Roots and leaves, moreover, had different proportions of individual ginsenosides. The most abundant ginsenosides were Rb_1 (56 mg · g^{-1} for Ontario; 37 mg · g^{-1} for British Columbia) and Re (21 mg · g^{-1} for Ontario; 15 mg · g^{-1} for British Columbia) in roots, and Rd (28-38 mg · g^{-1}), Re (20-25 mg · g^{-1}), and Rb_2 (13-19 mg · g^{-1}) in leaves. Measurable quantities of Rf were found in leaves (0.4-1.8 mg · g^{-1}) but not in roots or stems. Our results show that ginsenoside profiles in general, and Rf in particular, could be used for chemical fingerprinting to distinguish the different parts of the ginseng plant, and that ginseng leaves could be valuable sources of the ginsenosides Rd, Re, and Rb_2.

Key words : North American ginseng, *Panax quinquefolius,* Araliaceae, ginsenosides, plant parts, plant age, quantitative variation

INTRODUCTION

Recent increased interest in the use of alternative medicines in the prevention and treatment of chronic diseases and for health benefits has brought ginseng [*Panax quinquefolius* L. (North American ginseng) and *Panax ginseng* C. A. Meyer (Asian ginseng)] into the nutraceutical spotlight. Ginseng is a perennial plant belonging to the Araliaceae family, and has long been used in traditional medicine because of its supposed beneficial effects on physical health and mental state (e.g. alertness and power of concentration), especially in the elderly and those recovering from illness.[1,2] Several pharmacological investigations have reveal-ed multifaceted biological functions of ginseng, such as its salutary effects on the cardiovascular, immune, and nervous systems, and its role as an antidote, and an anti-cancer, anti-ageing, and anti-diabetic agent.[1, 3, 4]

The major bioactive constituents present in ginseng are dammarane saponins, commonly referred to as ginsenosides. Ginsenosides are glycoside derivatives of the triterpene dammarane. Over 30 ginsenosides have been isolated from roots, leaves, and flower buds of ginseng. The major ginsenosides are designated as R_0, Rb_1, Rb_2, Rc, Rd, Re, Rf, Rg_1, pseudoginsenoside F_{11}, and gypenoside XVII (Fig. 1).[5] The sugar moieties found in ginsenosides include glucose, arabinose, xylose and rhamnose.

Two of the principal ginseng species are *P. ginseng* C.A. Meyer and *P. quinquefolius* L., which are recognised for their commercial importance. There are also several minor species, including, *P. pseudoginseng* (Himalayan ginseng), *P. japonicus* C.A. Meyer, and *P. trifolius*.[6] The ginsenoside profiles of *Panax* species differ.[5] For instance, in the roots of *P. ginseng*, ginsenosides Rb_1, Rb_2, Rc, Re, Rg_1 and Rf are abundant, but pseudoginsenoside F_{11} is absent or present only in trace amounts.[5,7] In *P. quinquefolius,* Rf is absent in roots,[5,7,8] whereas, pseudoginsenoside F_{11} is present.[7] The objectives of this study were to measure major ginsenosides in different parts of the *P. quinquefolius* plant, and to study the concentration of ginsenosides as

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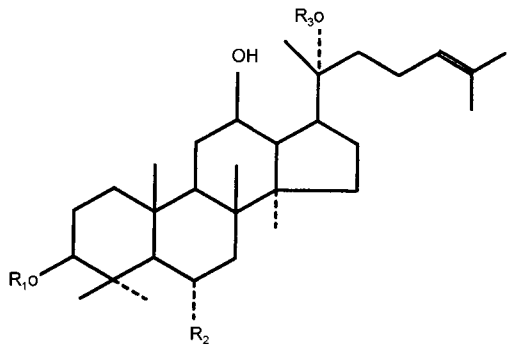
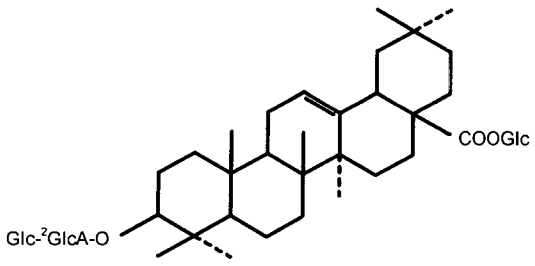


Ginsenoside	R_1	R_2	R_3
Rg_1	H	Glc-O	Glc-
Re	H	Rha-^{2}Glc-O	Glc-
Rf	H	Glc-^{2}Glc-O	H
Rb_1	Glc-^{2}Glc	H	Glc-^{6}Glc-
Rc	Glc-^{2}Glc	H	Ara(f)-^{6}Glc-
Rb_2	Glc-^{2}Glc	H	Ara(p)-^{6}Glc-
Rd	Glc-^{2}Glc	H	Glc-
Gypenoside XVII	Glc	H	Glc-^{6}Glc

Glc : glucose, Rha:rhamnose, Ara(f) : α-L-arabinofuranose, Ara(p) : α-L-arabinopyranose



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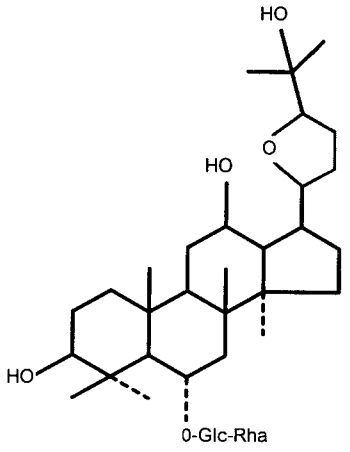

Pseudo Ginsenoside F_{11}

Fig. 1. Chemical structures of 10 major ginsenosides present in *P. quinquefolius* L.

functions of age of plant and two locations.

MATERIALS AND METHODS

1. Plant Materials and Chemicals

Whole ginseng plants (*P. quinquefolius* L., Araliaceae) of different ages were collected from commercial growers in Ontario and British Columbia during July and August of 1997 and 1998 (Specimen on file at OAC Herbarium at the University of Guelph, accession number is 8400). Some of the Ontario plants were grown in localities that included wooded area. Standard compounds, Rg_1, Rf, Rb_1, Rc, Rb_2, Rd, and Re were purchased from Indofine Chemical Company, Inc. (Somerville, New Jersey) and Ro, pseudoginsenoside F_{11}, and gypenoside XVII were generously provided by the Southern Crop Protection and Food Research Centre, Agriculture and Agri-Food Canada (London, Ontario, Canada).

2. Extraction and Analysis of Ginsenosides

The analytical method for ginsenosides was developed by modification of previously described methods.[9,10] Ginseng

Table 1. Content of total ginsenosides in roots, leaves and stems in relation to age of ginseng plant and growing location (Ontario and British Columbia)

Growing location	Plant part	Age of plant (years)			
		1	2	3	4
		Total ginsenoside concentration (mg/g DW)			
Ontario	Roots	13	33	71	98
	Leaves	72	81	61	86
	Stems	18	33	13	19
British Columbia	Roots	32	46	63	70
	Leaves	86	52	79	76
	Stems	37	17	16	19
SEM = 1.51; d.f = 70; n = 4					
Significance: L*, P**, Y**, L×P*, L×Y**, P×Y**, L×P×Y**					

*, **Significant at $P \leq 0.01$ or 0.001, respectively, where L=location, P=plant part and Y=plant age.

samples were dried at 50°C for 48 hrs, pulverized, and then passed through a 50-mesh screen. One hundred mg of ginseng powder was extracted by sonication with 4 mL of 80 % methanol and 20% 0.45 M KOH (1:1, v/v) at 50°C for 1 hr. The extracts were then neutralized using 350 μL of 14% KH_2PO_4, and the volume was made up to 10 mL. The solutions were centrifuged at 2,000 rpm for 10 min and an aliquot of supernatant was filtered through a 0.22-μm nylon filter. The ginsenosides were determined by high performance liquid chromatography (HPLC) using a Waters Symmetry C_{18} column (3.5 μm; 4.6x100 mm) on a Waters Millennium32 HPLC system with a Waters 660E system controller and Waters 717 autosampler employing a gradient solvent system. The solvent system consisted of (A) acetonitrile and (B) phosphate buffer (0.45 mM KH_2PO_4, pH 5.8) which were introduced into the column in accordance with the following protocol: 0-15 min, 20-20.9% (A); 15-15.5 min, 20.9-26% (A); 15.5-31.5 min, 26-27% (A); 31.5-32 min, 27-30% (A); 32-50 min, 30-33% (A); 50-60 min, 33-70% (A); 60-65 min, 70-75% (A); 65-65.5 min, 75-20% (A); and 65.5-75 min, 20% (A). The ginsenosides in the eluants were determined at a UV wavelength of 204 nm using a Waters 996 photodiode array detector (PDA). A root sample was spiked with 100 μg of Rg_1 and Rb_2 (dissolved in 200 μL acetonitrile:water, 30:70 v/v) at the beginning of the extraction procedure for calculation of analyte recovery. The samples were quantified using an external standard of each ginsenoside. Validation of the analytical method was performed at the ISO 9001:2000 registered Laboratory Services, University of Guelph, Ontario, Canada.

3. Statistical Analysis

The total concentrations of ginsenosides in different parts of plants (roots, leaves, and stems) from the two locations (Ontario and British Columbia) at ages of 1, 2, 3, and 4 years were tabulated in a 2×3×4 factorial arrangement (Table 1) employing a completely randomised design with four replicates. A replicate comprised parts of a representative ginseng plant produced by a selected grower at one or other of two growing locations. Analysis of variance was performed using version 8e of the SAS® system to test the main factor effects and their interactions.

RESULTS AND DISCUSSION

The content of ginsenosides in roots of *P. quinquefolius* increased significantly with the age of plant (Table 1 and Fig. 2). The total ginsenoside content of four-year-old roots from the two locations was 70 and 98 mg ginsenosides/g dry weight (DW) in British Columbia and Ontario respectively. This is comparable to data from Court *et al.*,[9] who reported that the total ginsenoside content increased from approximately 3% for first year roots to almost 8% for 4-year-old roots. The total ginsenosides in first year leaves were as high as those in roots of four-year-old plants, and the levels were relatively constant regardless of the age of plant. The total ginsenoside content of Ontario grown leaves ranged from 72 to 86 mg/g DW over the 4-year period (Table 1), although roots and leaves differed in their concentrations of individual ginsenosides. Li and Mazza[11] reported that the total ginsenoside content of leaves was higher than that reported in roots harvested from the same locations. Stems of the plant had relatively low levels of ginsenosides (13 to 37 mg/g DW) and did not increase with age. Some unexplained variation of ginsenoside content in stem and leaf of British Columbia ginseng was observed in year 2 (Table 1). The berries,

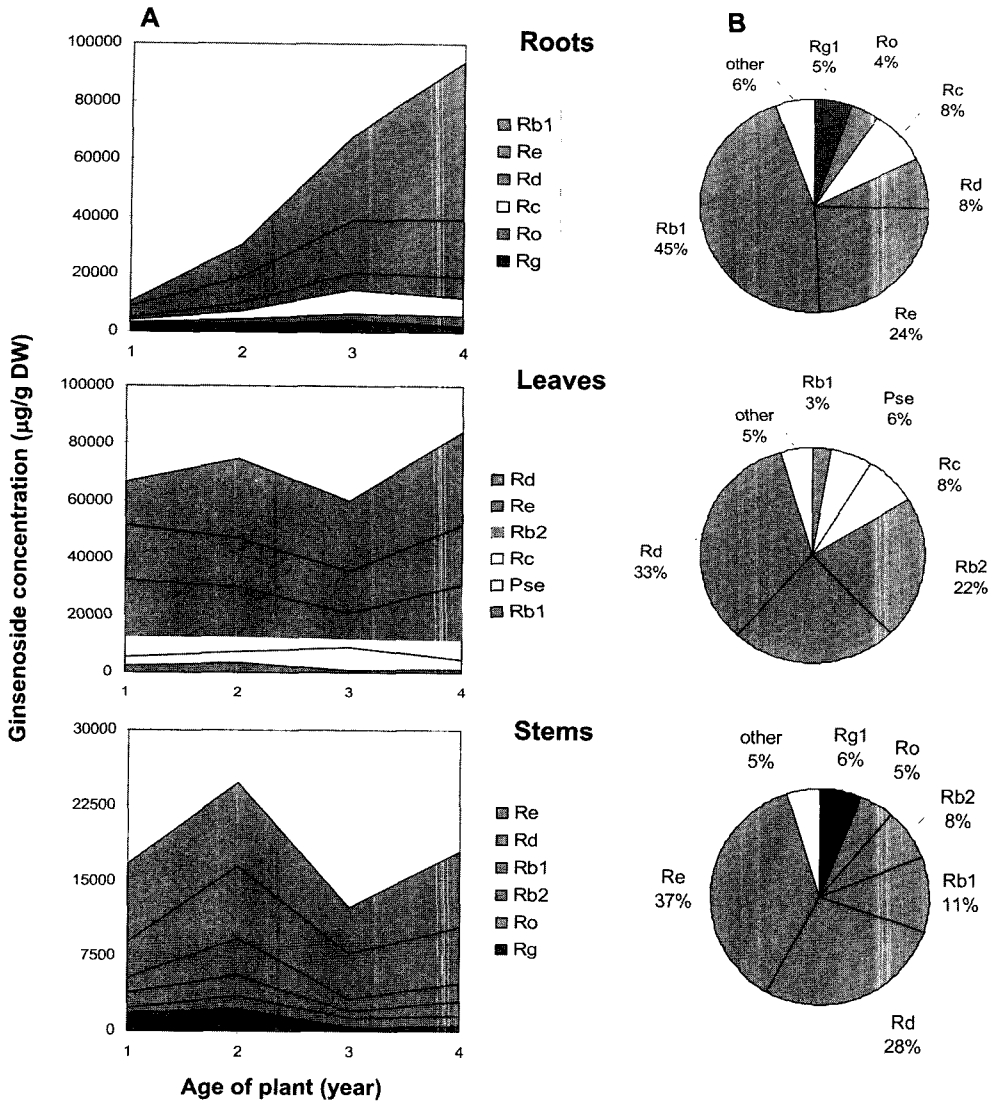

Fig. 2. Six major ginsenosides found in different plant parts in terms of (A) concentration pattern over the 4-year growing period and (B) percentage content in year 4.

which form during and after the third year of growth, had 13 to 26 mg/g DW (data not presented).

This study shows that the total ginsenosides in roots, leaves, and stems of *P. quinquefolius* differed appreciably (Table 1, Fig. 2 and 3). However, considering that the ginseng samples all belonged to the same species (*P. quinquefolius* L), but grown in different locations, the distribution pattern of the ten ginsenosides can be regarded as an inherent characteristic of the plant parts (roots, stems, and leaves) (Fig. 3). The total ginsenoside levels varied greatly between locations (Table 1), probably due to the genotypic effect or/and to differences in environmental conditions such as soil fertility, moisture, light quality and quantity, and use of agro-chemicals. The ginsenoside Rf was not detectable in roots of *P. quinquefolius* collected from multiple growing locations in Ontario and British Columbia. It was reported previously that the presence of Rf in roots is a unique characteristic of *P. ginseng* and the suggestion was made that Rf could therefore be used as a chemical marker to distinguish between roots of *P. ginseng* and *P. quinquefolius*.[5,7] We found, however, that Rf is detectable in leaves of *P. quinquefolius* even though it was not present in the roots (Fig. 3). Similarly, Li and Wardle[12] reported the presence of minute quantities of Rf in leaves and in roots. This contradiction in the literature could be due to the differences in the limits of determination for different analytical methods. Chan *et al.*[13] claimed that Rf and pseudoginsenoside F_{11} have the

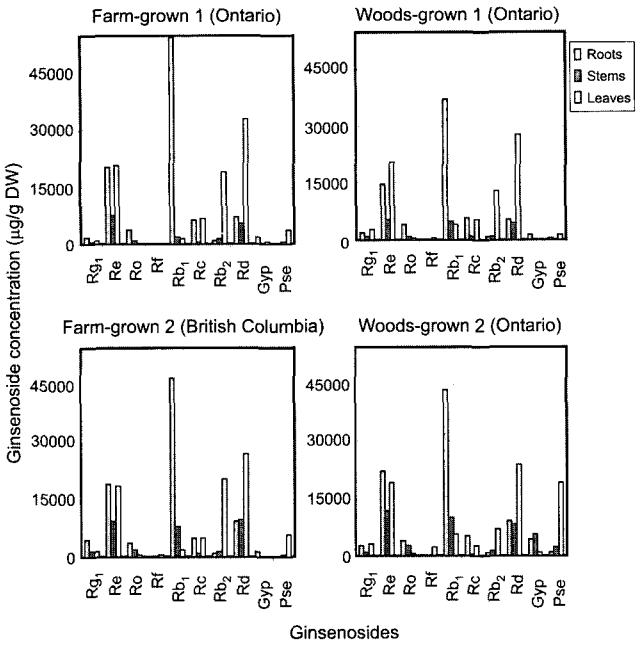

Fig. 3. Ginsenoside profiles of 4-year-old ginseng roots, stems and leaves in relation to different growing conditions/ locations.

same molecular weight and could therefore co-elute under most liquid chromatographic conditions. Under the present chromatographic conditions, authentic compounds of Rf and pseudoginsenoside F_{11} were well separated (with retention times of 30 and 58 min, respectively) and misidentification of these components is unlikely. Further studies to confirm the presence of Rf in leaves of *P. quinquefolius* are warranted.

In roots, Rb_1 and Re were the most abundant ginsenosides, accounting for about 79% of total ginsenosides present in 4-year roots (Fig. 2). Similarly, Li *et al.*[10] reported that Rb_1 and Re in roots of 4-year-old *P. quinquefolius* make up >75% of the total ginsenoside content. The abundance of Rb_1 and Re in roots is consistent in both farm- and woodland-cultivated *P. quinquefolius*.[8] The concentrations of Rb_1, Re, Rd, and Rc in roots increased up to a plant age of three years (Table 1, Fig. 2). Rb_1 increased greatly (23-46%) with age, even after 3 years compared with all other ginsenosides present in roots (Fig. 2). Court *et al.*[14] also observed that Rb_1 and Re are among the major ginsenosides in *P. quinquefolius*, increasing with age of roots.

In the 4-year-old ginseng plants from both Ontario and British Columbia, Rb_1 was 52 to 56% of ginsenosides in roots but only 1.6 to 5.1% in leaves. The three major ginsenosides present in leaves were Rd (37-38%), Re (24-27%), and Rb_2 (17-22%). Leaves contained 1.2-3.4 mg/g DW of the minor pseudoginsenoside F_{11} (1.7-4.0% of total ginsenosides) (Fig. 2 and 3). This compound is thought to enhance memory performance in people who consume *P. quinquefolius* (North American ginseng) [15]. It is important to note that roots of Asian ginseng (*P. ginseng*) contain only trace amounts of pseudoginsenoside F_{11} (less than 0.0001%).[7] Ginsenoside composition changes, and concentrations in leaves increase, with leaf maturity [9]. However, Starratt *et al.*[16] found that levels of ginsenosides in leaves of 3-year-old plants remained constant throughout the growing season, even with approaching senescence. Therefore removal of leaves at the end of the growing season may not affect the concentration of ginsenosides in roots.

In stems, Rd (23-30%) and Re (29-40%) predominated over other ginsenosides. As in leaves, the most abundant ginsenosides in berries were Re (34%), Rb_2 (33%) and Rd (14%) (data not presented). The concentrations of ginsenosides in leaves and stems, unlike those in roots, were independent of plant age (Table 1, Fig. 2).

Our research revealed that considerable quantities of ginsenosides are present in ginseng leaves as well as in the root, which, traditionally, is used for medicinal purposes. Moreover, the ginsenoside concentrations in leaves are high throughout the 4-year lifetime of the plant, whereas the concentrations in roots are relatively low initially and increased over time. Thus, the leaves of ginseng could be valuable sources of the ginsenosides Rd, Re, and Rb_2 for pharmacological studies and the nutraceutical industry in the future. The results indicate that ginsenoside profiles in general, and Rf in particular, could be used for chemical fingerprinting to distinguish the different parts of the ginseng plant and to differentiate between *P. quinquefolius* and *P. ginseng*. As shown, in *P. quinquefolius*, Rf is absent and pseudoginsenoside F_{11} is present in roots, whereas, in *P. ginseng*, Rf is abundant, but pseudoginsenoside F_{11} is absent or present only in trace amounts.

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